

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁴ : A01C 1/06		A1	(11) International Publication Number: WO 85/02972 (43) International Publication Date: 18 July 1985 (18.07.85)
(21) International Application Number: PCT/US85/00045 (22) International Filing Date: 14 January 1985 (14.01.85) (31) Priority Application Number: 570,611 (32) Priority Date: 13 January 1984 (13.01.84) (33) Priority Country: US		(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FR (European patent), GB (European patent), JP, LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report.</i>	
(71) Applicant: PLANT GENETICS, INC. [US/US]; 1930 Fifth Street, Davis, CA 95616 (US). (72) Inventors: REDENBAUGH, M., Keith ; 2816 Ganges Avenue, Davis, CA 95616 (US). REYES, Zoila ; 317 Yale Road, Menlo Park, CA 94025 (US). (74) Agents: WESEMAN, James, C. et al.; Limbach, Limbach & Sutton, 2001 Ferry Building, San Francisco, CA 94111 (US).			

(54) Title: COATING HYDROGEL CAPSULES

(57) Abstract

Methods and materials for coating hydrogel capsules to control the migration of solutes or solvents between the capsule and the environment. The membranes may be of a single compound or various compounds which provide control over the migration of distinct elements.

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT Austria	GA Gabon	MR Mauritania
AU Australia	GB United Kingdom	MW Malawi
BB Barbados	HU Hungary	NL Netherlands
BE Belgium	IT Italy	NO Norway
BG Bulgaria	JP Japan	RO Romania
BR Brazil	KP Democratic People's Republic of Korea	SD Sudan
CF Central African Republic	KR Republic of Korea	SE Sweden
CG Congo	LI Liechtenstein	SN Senegal
CH Switzerland	LK Sri Lanka	SU Soviet Union
CM Cameroon	LU Luxembourg	TD Chad
DE Germany, Federal Republic of	MC Monaco	TG Togo
DK Denmark	MG Madagascar	US United States of America
FI Finland	ML Mali	
FR France		

Description
Coating Hydrogel Capsules

Technical Field

This invention relates to the production of
5 membranes for coating hydrogel capsules, and more
particularly to membranes which control solute and
solvent migration between the capsule and its
environment.

Background of the Invention

10 A conventional technique of coating hydrogel
capsules is to construct a polylysine membrane around
the capsule. See F. Lim, U.S. Patent 4,352,883. Other
techniques involve methylcellulose compounds, polyvinyl
alcohols, plastics, and other compounds. See L.
15 Lachman, H.A. Lieberman and J.L. Kanig, eds. "The
Theory and Practice of Industrial Pharmacy," Lea and
Febiger, Philadelphia, PA, 1970, p. 197-225.

However, conventional membranes do not control the
passage of solvents, particularly water. An additional
20 drawback is the failure to control the passage of small
molecular weight solutes across the membrane, unless
the membrane is rigid and virtually impermeable.

The above limitations are important when the
capsule to be coated is a hydrogel capsule intended to
25 contain living material, notably botanic tissue, such
as seeds, somatic embryos and other meristematic
tissue. In these applications the membrane must allow
respiration by the tissue contained in the capsule,
thus an impermeable capsule is undesirable. Yet it is
30 important that the capsule retains sufficient moisture
and solutes for tissue viability. Furthermore, the
capsule and membrane are often desirably a temporary
container for encapsulated tissue, and they will

deteriorate or fracture at the desired time for emergence of the tissue, such as e.g. germination.

In addition to the above limitations with available capsule coatings, in some applications it is 5 desirable to provide a coated capsule which is not cohesive or adhesive, as this facilitates handling and bulk storage.

Disclosure of the Invention

Thus, an object of this invention is to provide 1. methods and compositions which control the passage of solvents between hydrogel capsules and their environment.

Another object of the invention is to provide methods and compositions whereby the passage of solutes 15 into and out of hydrogel capsules is controlled.

Yet another object of the invention is to provide coatings around hydrated capsules which will break down under septic conditions.

A further object of this invention is to provide 20 hydrogel capsules which can be handled as flowable, non-adherent units.

An additional object of the invention is to provide a method whereby coated hydrogel capsules can be stored for an extended time period without 25 substantial water loss from the capsule.

In accordance with the invention, improved methods and membranes are provided for coating hydrogel capsules used for encapsulating material. One aspect of the invention provides a method for encapsulating 30 material and separating the material from its environment comprising encapsulating the material in a hydrogel capsule and surrounding the capsule with at least one membrane which controls the migration of solutes or solvents between the capsule and the 35 environment.

Another aspect of the invention provides a capsule membrane which comprises at least one coating surrounding the capsule which reduces the flow of solvents or solutes between the capsule and its environment.

5 Best Mode for Carrying Out the Invention

Briefly, in accordance with the invention, hydrogel capsules are produced with controlled-release membranes by coating the capsules with specific polymers, binders and solvents.

10 The invention provides specific polymers and other compounds which are hydrophobic and therefore prevent passage of water when the compounds are formulated as sheets or films. By controlling the passage of water, the present invention also controls the passage of
15 aqueous solutes.

The hydrophobic membranes provided will individualize the capsules and minimize capsules adhesions.

Desirably, in accordance with the invention, the
20 coating can break down at an appropriate point in time to release the capsule contents. The invention is particularly advantageous in creating a protective, solvent-controlling, controlled-release membrane around hydrogel capsules.

25 Selection of Hydrogels

Individual capsule particles may be produced from a wide variety of hydrogel polymers. Such gels in an uncoated state should allow acceptance, containment and release of specific solvents, solutes and other
30 adjuvants as well as diffusion of gases. The gel should provide an environment strong enough to resist external abrasion and adverse forces, yet pliable enough to allow release of internal components and breakdown of the hydrogel at the appropriate time. It

may be desirable to use various gels in combination, either as a mixture, as layers or as discrete regions to achieve the desired results.

Hydrogel capsules made from sodium alginate 5 complexed with calcium contain approximately 90% water. When stored, the water gradually evaporates because calcium alginate is permeable to water. When stored under refrigeration, this water collects in the container, causing the capsules to adhere to the 10 container and each other.

Gels which have been found useful for encapsulating solvents, solutes and other adjuvants include sodium alginate, guar gum, carrageenan with locust bean gum, sodium alginate with gelatin, carboxymethylcellulose, gum tragacanth, sodium pectate and vinyl acetate. 15 Other suitable gels include, but are not limited to:

TABLE 1. GEL AGENTS

I. Natural Polymers

A. Ionic bonds (requires complexing agents)

20 Alginate with Gelatin

Sodium Pectate

Furcellaran

Pectin

Hypnean

25 Dextran

Tamarind

Guar Gum

B. Hydrophobic Interactions

30 Amylose

Agar

Agarose

Agar with Gelatin

Gelatin

Starch

35 Amylopectin

- Cornhull Gum
Starch Arabogalactan
Gum Ghatti
Gum Karagan
5 Ti Gum
Gum Tragacanth
Wheat Gum
Chitin
Dextrin
- 10 II. Chemically Modified Natural Polymers
A. Ionic bonds (requires a complexing agent)
Ethyl Succinylated Cellulose
Succinylated Zein
Carboxymethylcellulose
- 15 B. Hydrophobic Interactions
Methylcellulose
Hydroxyethyl Cellulose
- C. Covalent Bonds
Gelatin with Glutaraldehyde
- 20 III. Synthetic Polymers
A. Covalent Bonds
Polyacrylamide
- B. Hydrophobic Interactions
Polyethylene Glycol
25 Polyvinylpyrrolidone
Polyoxyethylene
Hydrophilic Urethane
Polyvinylacetate
Vinyl Resins
- 30 Hydron (hydroxyethylmethacrylate)
2-methyl-5-vinylpyridine-
methylacrylate-methacrylic acid
- C. Ionic Bonds
35 Sodium poly (styrene sulfonate) with
poly(vinyl methyl pyridinium) chloride
Sodium poly (styrene sulfonate) with poly ,

(vinyl benzyl trimethyl ammonium) chloride
Strongly acidic polyanion with strongly basic
polycation

5 Bordon Poly Co. 2113® (vinyl acetate
homopolymer) (Bordon Co.)

Gelvatol® (polyvinyl alcohol resin)(Monsanto)

IV. Stabilizing Compounds

A. Trade Names

10

Super Slurper® (USDA, SEA-AR,
Nor. Reg. Res. Lab)

Viterra® (Union Carbide)

Laponite® (Laporte (United States) Inc.)

Gelrite® (Kelco)

SeaKem® (FMC Corporation)

15

SeaPlaque® (FMC Corporation)

SeaPrep® (FMC Corporation)

IsoGel® (FMC Corporation)

B. Organic Compounds

20

Methylan Clear Wallpaper Paste

Lactose

Wax

Protein Colloids

C. Inorganic Compounds

25

1. Clay

2. Compounds which adhere by means
of a water-soluble plastic
such as methylcel:

Fly Ash

Feldspar

30

Celrite

Bentonite

Vermiculite

Diatomaceous Earth

Lime

35

Calcium Carbonate

3. Other

Calcium Oxide
Magnesium Carbonate
Sodium bicarbonate
Urea

5 Encapsulation with Selected Gel

Once the gel has been chosen, there are numerous parameters which influence the characteristics previously mentioned.

A sodium alginate solution, for example, will form 10 a gel when a complexing agent is added. Calcium chloride (CaCl_2) is generally used, however, lanthanum chloride, ferric chloride, cobaltous chloride, calcium nitrate, calcium hydroxide, superphosphate fertilizer, and many pesticides such as benefin, alachlor and 15 chlorpropham are also acceptable, as are other multivalent cation compounds.

An important factor which influences the choice of 20 gel agent, and other parameters, is the choice of encapsulated material. In general the coated hydrogel capsules of the present invention will find use as delivery systems for, e.g. living material including eukaryotic cells, micro-organisms and botanic tissue such as seeds, somatic embryos and other meristematic tissue capable of developing into an entire plant body. 25 Alternatively, numerous adjuvants may be encapsulated within the capsules of the present invention, either separately or in combination with each other and with encapsulated living material.

A chosen gel will have a range of concentrations 30 usable in working the invention. A concentration should be chosen to optimize ease of handling, gelling time, strength of gel and coating thickness around the encapsulated material. If the gel is too dilute, the encapsulated material can settle during gel formation 35 and produce an uneven encapsulation.

The sodium alginate may be prepared in a concentration of 1 to 10% w(in grams)/v(in milliliters) in water, more usually 2 to 10% and ideally from 3 to 5%.

Specific adjuvants to be encapsulated may be mixed 5 with the sodium alginate at concentrations specific for the application rates of the particular adjuvants. The dispersed adjuvants in gel solution may then be added dropwise to the complexing agent. Alternatively, the gel solution and complexing agent may be mixed by any 10 of numerous techniques known to the art. These may include droplet formation and agent addition as a one step process by a vibrating nozzle which ejects a gel droplet from one source and coats the droplet with complexing agent from another.

15 The calcium chloride (or other complexing agent) may be made up in solution at a concentration of 1 to 1,000 millimolar, more usually 20 to 500 millimolar and ideally from 50 to 300 millimolar. Other complexing agents will have different preferred concentration 20 ranges.

The time for gel formation and temperature of the gelling solutions are interrelated parameters, for selected concentrations of gel and complexing agent. The temperature should be chosen in the range of 1 to 25 50°C, more usually 10 to 40°C, and preferably at 20 to 40°C.

Within the range of acceptable temperatures, a particular value may be chosen to give the shortest possible gelling time consistent with complete gel 30 formation. Typically, the gel will form immediately, but the complexation takes much longer. For a solution of sodium alginate at a concentration of 3.2 grams per 100 milliliters H₂O, calcium chloride solution concentration of 50 millimolar, and 25°C reaction 35 temperature, adequate gelling is obtained in 5 to 120 minutes, more often 10 to 90 minutes and is usually

sufficiently complete in 30 to 60 minutes.

Alternatively, if 300 millimolar calcium chloride is substituted for 50 millimolar calcium chloride, gelation time is decreased to 2-5 minutes.

5 The gel characteristics described above are modifiable for each gel, but are determined generally by the concentration parameters and chemical properties of the gel.

10 Coating the Capsules with Solvent/Solute Controlling Membrane

Subsequent to capsule formation, it may be desirable to control permeability of the outer surface of the gel matrix. The encapsulated adjuvants can be coated with a membrane resistant to water or solvent movement. The membrane should be semipermeable to impede the release of the capsule contents or impermeable to the contents with release being effected via microbial degradation, temperature or pH changes, or other physical or biological effects. This membrane can also influence the handling properties of the capsules, particularly the flowability of the capsule.

The coating materials selected to provide membranes for hydrogel capsules will ideally produce membranes with these desirable properties:

- 25 1. The membrane will be relatively water-impermeable.
2. The membrane will adhere to the hydrogel capsule.
3. The membrane will optionally be biodegradable.
- 30 4. The solvent systems (if necessary) and procedures used to apply the membrane will be relatively harmless to the material encapsulated by the hydrogel.

35 In general, hydrophobic substances can be used as water-impermeable membranes when used separately, as

mixtures or when combined with polymeric binders, such as e.g. ethylene-vinyl acetate copolymers, and cellulose derivatives.

For example, various polymers such as Elvax 4260®
5 (ethylene vinyl acetate acrylic acid terpolymer,
DuPont, Wilmington, DE) will form coatings resistant to
water penetration when mixed with stearic acid, cetyl
alcohol, cyclohexane and petroleum ether. Capsules are
pretreated with a calcium oxide solution and immersed
10 in the polymer solution. In a similar manner other
polymers can be used to coat capsules.

A chosen polymer will have a range of concentrations usable in working the invention. A concentration should be chosen to optimize ease of
15 handling, viscosity, solubility and membrane thickness. If the polymer concentration is too low, the membrane will be readily permeable to water. If too thick, the optionally included meristematic tissue may not germinate.

20 The Elvax 4260® may be prepared in a concentration of 1 to 50% w(in grams)/v(in grams) in cyclohexane, more usually 5 to 20% and preferably 8 to 12%. One gram of this Elvax solution is then mixed with stearic acid, cetyl alcohol, and petroleum ether (60-110°C
25 boiling point) at concentrations of optionally .01 to 5.0 grams, .01 to 5.0 grams and .1 to 20 grams, respectively, more usually .1 to 1.0, .1 to 1.0 and .5 to 10.0 grams, respectively to produce a polymer solution. Capsules containing various material and
30 adjuvants are pretreated with calcium hydroxide at a concentration of .001 to 1.0 grams per gram water, more usually 0.01 to 0.1 grams per gram. The pretreatment solution is applied to the capsules at a concentration of 0.5 to 20.0 grams pretreatment solution per gram
35 capsules, more usually 1 to 10 grams per gram. Following pretreatment, the capsules are immersed and

stirred for 1 to 30 minutes, more usually 3 to 10 minutes in the polymer solution at ambient temperature, 20 to 35°C, more usually 25 to 30°C. Subsequently, the capsules are stirred 1 to 30 minutes, more usually 3 to 5 10 minutes at 0 to 10°C, more usually 3 to 8°C. The capsules are filtered to remove excess polymer solution and then air dried.

As an alternative to the pretreatment protocol, .0001 to 1.0 gram each of glucose and glycerol, more 10 usually .01 to .10 gram each are added to the calcium hydroxide-solution before capsules are pretreated.

As an alternative to the polymer solution, Spermaceti Wax Substitute #573 (J. B. Ross Co., Jersey City, N.J.) can be added to the Elvax 4260® solution at 15 a concentration of .001 to 2.0 gram per gram Elvax 4260® solution, more usually .01 to 1.0 gram per gram.

As a further alternative, the polymer solution may be replaced with a solution of 0.05 to 10.0 grams, more usually 0.5 to 5.0 grams of a 1 to 20%, more usually 5 20 to 15% Elvax 310® (ethylene vinyl acetate copolymer) in high boiling point petroleum ether mixed with .001 to 2.0 grams each of stearic acid and cetyl alcohol, more usually 0.01 to 1.0 grams each.

As another alternative, the polymer solution may 25 be replaced with 0.01 to 10.0 grams, more usually 0.1 to 5.0 grams of .50 to 10%, more usually 1 to 5% aluminum monostearate in toluene mixed with .001 to .10 grams, more usually .01 to .10 grams stearic acid.

After applying this coating, a second coating may be 30 applied consisting of 0.01 to 10.0 grams, more usually 0.1 to 5.0 grams of .5 to 10%, more usually 1 to 5% aluminum tristearate in toluene mixed with .01 to 5.0 grams, more usually .10 to 1.0 grams of .50 to 20%, more usually 5 to 15% Elvax 4260® in cyclohexane.

35 As yet another alternative, the polymer solution may be replaced with .10 to 10 grams, more usually .50

to 2.0 grams of methylvinyl ether/maleic anhydride copolymer, high molecular weight, dissolved in 5 to 100 milliliters, more usually 10 to 50 milliliters of anhydrous butanol. One drop of sulfuric acid is added 5 to the mixture. This solution is refluxed for 20 to 30 hours at 100 to 120°C.

As an additional polymer alternative, Gantrez ES-435® or ES-425® (GAF Corporation) may be used.

Other coating compounds which can be used in the 10 present invention to form membranes include, without limitation: .

TABLE 2. CAPSULE COATING COMPOUNDS

I. Enteric Coating

- Methylvinyl ether/maleic anhydride
15 Styrene maleic acid copolymer
Styrene-maleic anhydride copolymer
Ethylene/maleic anhydride copolymer

II. Hydrophobic Polymers

- Ethylcellulose
20 Isopropyl myristate
Polyvinyl acetate phthalate
Starch acetate phthalate
Amylose acetate phthalate
Cellulose acetate phthalate

25 Saran

Butyl rubber

III. Other Compounds

- Keratin
Shellac
30 Carnuba wax
Paraffin
Wax
Fats
Lipids

Triglycerides
Ethylene vinyl acetate copolymer
Benzyl cellulose
Petrolatum

5 As an alternative to immersing the capsules in the polymer solution numerous procedures for applying the coating compound to form a hydrogel capsule membrane were used.

Spraying

10 For this coating procedure an inlet tube was inserted into the lower side of a stainless steel beaker and the beaker covered with a polypropylene mesh attached to the nozzle of a spray gun. The cover was fastened to the beaker with a rubber band. This 15 apparatus simulates a pan-spray tablet coater. The procedure consisted of the following steps:

1. The capsules were placed in the beaker and the cover attached.
2. The capsules were sprayed with the solution of coating compound and the beaker agitated by hand to ensure uniform spraying.
3. The capsules were dried with pressurized air through the inlet tube, while agitating the beaker by hand.

25 Steps 2 and 3 were repeated until a suitable coating was applied or the solution was exhausted.

Because it was difficult to maintain adequate agitation during this coating process, a nylon mesh bag was substituted for the beaker. The bag was attached 30 to the nozzle, the capsules were alternately sprayed and dried while frequently shaking the bag to uniformly deposit the coating on the capsules.

Immersion

This procedure consisted of the following steps:

1. The capsules were immersed in the coating compound solution, generally at 30° to 35°C.
- 5 2. The mixture was stirred 5 min at room temperature and 5 min in an ice-water bath at 10°C.
3. The capsules, were filtered, rinsed with low-boiling petroleum ether, and dried with
- 10 pressurized air or nitrogen.

Dipping

This procedure consisted of the following steps:

1. The capsules were placed on a basket or bag. (A basket made of polypropylene mesh was used initially, but a bag made of a nylon mesh was found more convenient because of its flexibility.)
- 15 2. The bag with the capsules was dipped into the coating compound solution, and agitated to ensure that all the capsules were coated.
3. The bag was removed from the solution, and allowed to drain on a funnel.
- 20 4. The capsules were dried with a gentle flow of pressurized air.
5. Steps 2-4 were repeated three or four times and the capsules dried thoroughly with
- 25 pressurized air.

Experimental

In order to demonstrate the invention, without implying limitations, the following experiments were carried out with a variety of coatings. All quantities labelled percent (%) are grams per 100 milliliters, unless otherwise indicated.

Example A(Coating Capsules Containing Alfalfa Seeds)

500 alfalfa seeds, Saranac AR Lot #27-07-765
(Whitney Dickenson Seed Growers, Homedale, Idaho), were
5 mixed with 200 milliliters of 3.2% sodium alginate.
This solution was added drop-wise into 500 milliliters
of 100 millimolar calcium chloride to complex the
alginate and form spheroid capsules. 40 grams of the
capsules were stirred in 8 grams of lime water
10 (containing .20 grams calcium hydroxide plus 1 gram
each of glucose and glycerol) for one minute. The
solution was decanted and the capsules were immersed in
a 30°C polymer solution containing 2 grams stearic
acid, 2 grams cetyl alcohol, 10 grams of a 10% solution
15 of Elvax 4260® (ethylene vinyl acetate acrylic acid
terpolymer) in cyclohexane, and 40 grams of petroleum
ether. The capsules were stirred for 5 minutes at room
temperature followed by 5 minutes more in an icewater
bath. The capsules were filtered through a nylon
20 cloth, rinsed with 5 milliliters petroleum ether (30-
60°C), and dried with pressurized air. Subsequent to
coating the encapsulated seeds, the alfalfa seeds
imbibed water from the alginate capsule, germinated,
and emerged from the capsules undamaged.
25 A.1. As an alternative polymer solution, 40 grams of
2% aluminum monostearate in toluene plus 2 grams
stearic acid can be used.
A.2. As another alternative polymer solution, 5.2
grams of spermaceti wax substitute #573, 2 grams
30 cetyl alcohol, 1 gram stearic acid, 10 grams of a
10% solution of Elvax 4260® in cyclohexane, and
40 grams of high boiling point petroleum ether
can be used.
A.3. As a further alternative polymer solution, 4
35 grams of stearic acid, 4 grams of cetyl alcohol,
and 40 grams of a 10% solution of Elvax 310®

(ethylene vinyl acetate copolymer) in high boiling point petroleum ether can be used.

A.4. As another alternative polymer solution, 2 grams of stearic acid plus 40 grams of a 2% solution of aluminum monostearate in toluene can be used as a first coating. A second coating can then be applied consisting of 32 grams of a 2% solution of aluminum tristearate in toluene mixed with 10 grams of a 10% solution of Elvax 4260® in cyclohexane.

A.5. As a still further alternative, 10 grams of methylvinyl ether/ maleic anhydride copolymer was refluxed in 50 milliliters of anhydrous butanol for 21 hours at 110°C. Subsequently, one drop of sulfuric acid was added to the solution. The solution was mixed with alginate-encapsulated alfalfa seeds and dropped into water to produce coated capsules.

A.6. As yet another alternative polymer, Gantrez ES-435® or ES-425® (GAF Corporation) was mixed with alginate beads and dropped into water to form coated capsules.

Example B

(Coating Capsules Containing Tomato Seeds)

Six hundred tomato seeds, UC-82 (VGY9225, Asgrow), were encapsulated in 2% w/v sodium alginate by dropwise complexation in 100 millimolar calcium chloride. Three hundred of these capsules were stirred one minute in 20 grams of a calcium hydroxide solution containing 1 gram of glucose and 2 grams of glycerol. The calcium hydroxide solution was prepared by stirring 1 gram of calcium hydroxide in 100 milliliters of water for 15 minutes and then filtering the solution. The treated capsules were sieved through a nylon screen, patted dry, and dipped 6 times for 1-3 seconds each in the

- membrane coating solution. The capsules were blow-dried between dippings. The membrane coating solution consisted of the combination of the following 3 solutions: 2 grams Elvax 4260® dissolved in 20 grams 5 cyclohexane, 10 grams of spermacetti wax substitute #573 melted with 4 grams cetyl alcohol and 2 grams stearic acid, and 80 grams each of petroleum ether (50-110°C) and methylene chloride. Capsules were planted under both greenhouse and field conditions.
- 10 The membrane coated, encapsulated seeds had a 100% germination frequency in the greenhouse which was equal to the non-coated, encapsulated seeds and non-coated, non-encapsulated seeds. In the field, membrane-coated, encapsulated seeds had germination frequencies equal to 15 non-coated, encapsulated seeds and better than non-coated, non-encapsulated seeds.
- B.1. As an alternative to encapsulation, non-encapsulated seeds were coated as described. Germination rates of coated seeds in the 20 greenhouse equaled rates for non-coated seeds.

Example C

(Coating Capsules Containing Alfalfa Somatic Embryos)

1. Coating By Immersion

Alfalfa somatic embryos were produced as described 25 by K. A. Walker and S. J. Sato (Plant Cell Tissue and Organ Culture 1:109-121, 1981) using a regeneration medium consisting of Shenk and Hildebrandt (SH) medium (R. V. Shenk and A. C. Hildebrandt, Canadian Journal of Botany 50:199-204, 1972), 100 millimolar proline, and 30 25 millimolar ammonium. The somatic embryos were encapsulated as in Example A. The encapsulated embryos were then pretreated in a calcium hydroxide solution as in Example B followed by coating as in Example A. The coated, encapsulated somatic embryos were placed on 35 one-half strength SH medium and incubated at 27°C

under 16 hour light/8 hour dark conditions. The percent embryo viability, radicle emergence, and shoot/leaf emergence was statistically equal to that for non-coated, non-encapsulated somatic embryos and 5 for non-coated, encapsulated somatic embryos.

1.A. As an alternative, non-encapsulated alfalfa somatic embryos were coated. Percent embryo viability, radicle emergence, and shoot/leaf emergence was statistically equal to that for 10 non-coated somatic embryos.

2. Coating By Dipping

The experimental protocol C.1. was duplicated, substituting a dipping process for the immersion coating method. The encapsulated alfalfa somatic 15 embryos were placed in a nylon bag and dipped three times in the coating solution. The capsules were blow-dried and dipped three more times with a final blow-drying. The percent embryo viability, radicle, emergence, and shoot leaf emergence was statistically 20 equal to that for non-coated, encapsulated somatic embryos.

2.A. As an alternative, non-encapsulated scatic embryos were coated. Percent embryo viability, radicle emergence, and shoot/leaf emergence was 25 statistically equal to that for non-coated somatic embryos.

Example D (Capsule Storage)

Capsules without seeds were coated as in protocol 30 A and stored either in an open container or in a sealed vial. Initial water loss from the capsules during the coating process was 10% w/w of water per capsule. Subsequent water loss through the membrane for capsules stored in an open container was 0 over a 3-day period.

- B.1. As an alternative, a different sample retained 93% water over a 10-day period.
- B.2. As an alternative, a further sample retained 75% water over a 30-day period.

5 Although the foregoing invention has been described in some detail by way of illustration for purposes of clarity of understanding, it will be understood that numerous modifications may be practiced within the spirit and scope of the appended claims.

CLAIMS

1. A capsule membrane for hydrogel capsules encapsulating material and separating said material from its environment, which membrane comprises:

5 at least one coating surrounding the capsule which reduces the flow of solvents or solutes between the capsule and its environment.

2. A capsule membrane as recited in Claim 1 wherein the encapsulated material is living material.

10 3. A capsule membrane as recited in Claim 2 wherein the living material is plant meristematic tissue.

4. A capsule membrane as recited in Claim 1 or 3 wherein the capsule further comprises a gel matrix comprising at least one agent selected from the group consisting of sodium alginate, guar gum, carrageenan with locust bean gum, sodium alginate with gelatin, carboxymethylcellulose, gum tragacanth, sodium pectate, vinyl acetate and hydrogel agents identified in Table
20 I.

5. A capsule membrane as recited in Claim 1 or 3 wherein the membrane further comprising at least one compound selected from the group consisting of ethylene vinyl acetate acrylic acid terpolymers, ethylene vinyl acetate copolymers, spermaceti, aluminum stearates, methyl vinyl ether/maleic anhydride copolymers, ethyl cellulose, ethylhydroxyethyl cellulose, stearic acid, glyceryl monooleate, cetyl alcohol, calcium stearate and compounds identified in Table II.

6. A capsule membrane as recited in Claim 4 wherein the membrane further comprising at least one compound selected from the group consisting of ethylene vinyl acetate acrylic acid terpolymers, ethylene vinyl acetate copolymers, spermaceti, aluminum stearates, methyl vinyl ether/maleic anhydride copolymers, ethyl cellulose, ethylhydroxyethyl cellulose, stearic acid, glyceryl monooleate, cetyl alcohol and calcium stearate.
- 10 7. A capsule membrane as recited in Claim 1 or 3 wherein the capsule further comprises a gel matrix comprising at least one agent selected from the group consisting of sodium alginate, guar gum, carrageenan with locust bean gum, sodium alginate with gelatin, 15 carboxymethylcellulose, gum tragacanth, sodium pectate and vinyl acetate.

8. A capsule membrane as recited in Claim 1 or 3 wherein:

the capsule further comprises a gel matrix
20 comprising at least one agent selected from the group consisting of sodium alginate, guar gum, carrageenan with locust bean gum, sodium alginate with gelatin, carboxymethylcellulose, gum tragacanth, sodium pectate and vinyl acetate; and
25 the membrane further comprising at least one compound selected from the group consisting of ethylene vinyl acetate acrylic acid terpolymers, ethylene vinyl acetate copolymers, spermaceti, aluminum stearates, methyl vinyl ether/maleic anhydride copolymers, ethyl cellulose, ethylhydroxyethyl cellulose, stearic acid, 30 glyceryl monooleate, cetyl alcohol and calcium stearate.

9. A method for encapsulating material and separating the material from its environment comprising encapsulating material in a hydrogel capsule, and surrounding the capsule with at least one membrane 5 which controls the migration of solutes or solvents between the capsule and the environment.

10. A method as recited in Claim 9 wherein the encapsulated material is living material.

11. A method as recited in Claim 10 wherein the 10 living material is plant meristematic tissue.

12. A method as recited in Claim 11 further comprising encapsulating the material in the capsule and membrane of Claim 4.

13. A method as recited in Claim 11 further 15 comprising encapsulating the material in the capsule and membrane of Claim 5.

14. A method as recited in Claim 11 further comprising encapsulating the material in the capsule and membrane of Claim 6.

20 15. A method as recited in Claim 11 further comprising encapsulating the material in the capsule and membrane of Claim 7.

16. A method as recited in Claim 11 further 25 comprising encapsulating the material in the capsule and membrane of Claim 8.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US85/00045

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³

According to International Patent Classification (IPC) or to both National Classification and IPC ⁴

A01C 1/06

US 47/57.6

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System	Classification Symbols
47	57.6, Dig. 9

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁵

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁶

Category ⁷	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X,Y,P	USA4,434,231 28 May 1984 Jung	1-3
X,Y	USA4,378,367 29 March 1983 Brooker et al	1-3
X,Y	USA4,344,979 17 August 1982 Gago et al	1-3
X,Y	USA4,249,343 10 February 1981 Dannelly	1-3 and 9-11
X,Y	USA3,911,183 7 October 1975 Hinkes	1-3
Y	USA4,241,537 30 December 1980 Wood	4-8

* Special categories of cited documents: ¹⁴

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ¹⁹

4 April 1985

Date of Mailing of this International Search Report ¹⁹

16 APR 1985

International Searching Authority ¹

TGA/US

Signature of Authorized Officer ¹⁹

H. J. Hall

This Page Blank (uspto)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

This Page Blank (uspto)